Journal of the American College of Cardiology
© 2002 by the American College of Cardiology Foundation
Published by Elsevier Science Inc.

Vol. 40, No. 7, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(02)02174-5

Plasma Asymmetric Dimethylarginine and Hyperemic Myocardial Blood Flow in Young Subjects With Borderline Hypertension or Familial Hypercholesterolemia

Hannu Päivä, MD,* Juha Laakso, MSc,† Hanna Laine, MD, PhD,‡ Reijo Laaksonen, MD, PhD,*† Juhani Knuuti, MD, PhD,§ Olli T. Raitakari, MD, PhD§‖

Tampere, Helsinki, and Turku, Finland

OBJECTIVES The goal of this study was to examine the relationship between plasma asymmetric dimethylarginine (ADMA) level and hyperemic myocardial blood flow (MBF) in subjects with borderline hypertension (BHT) and familial hypercholesterolemia (FH).

BACKGROUND Asymmetric dimethylarginine is an endogenous competitive inhibitor of nitric oxide synthase that may modulate vascular function.

METHODS We measured plasma ADMA levels and myocardial flow in 77 young men (mean age 35 ± 5 years), including 47 healthy controls, 16 men with BHT, and 14 men with FH. Basal and dipyridamole-induced myocardial flow was measured using positron emission tomography. Plasma ADMA levels were measured using high-pressure liquid chromatography.

RESULTS Asymmetric dimethylarginine levels were significantly elevated in the BHT group compared with controls (0.59 ± 0.13 μmol/l vs. 0.43 ± 0.12 μmol/l, $p < 0.001$), and they had significantly lower dipyridamole flow (2.85 ± 1.20 ml/min/g vs. 3.69 ± 1.68 ml/min/g, $p < 0.05$). In a multivariate regression model adjusted for the study group, dipyridamole flow was inversely associated with ADMA ($p < 0.05$), age ($p < 0.05$), and apolipoprotein B concentration ($p < 0.05$).

CONCLUSIONS We conclude that plasma ADMA concentration is related to dipyridamole-induced vasodilatory function in young men, independently of blood pressure elevation and hypercholesterolemia. Subjects with BHT have significantly increased plasma ADMA levels, which may partly explain the impaired hyperemic MBF in this condition. (J Am Coll Cardiol 2002;40: 1241–7) © 2002 by the American College of Cardiology Foundation

Myocardial vasodilator capacity has been shown to become reduced in subjects free from clinical atherosclerosis in the presence of vascular risk factors, such as hypercholesterolemia (1–3), diabetes (4), elevated blood pressure (BP) (5), and increased lipoprotein oxidation (6). These findings suggest that abnormal hyperemic myocardial blood flow (MBF) may represent an early marker of coronary atherosclerosis. This is supported by our recent observations in healthy young adults showing a significant relationship between a structural marker of subclinical atherosclerosis, increased carotid artery intima-media thickness (IMT), and reduced myocardial flow response to dipyridamole (7). Dipyridamole-induced myocardial vasodilation is an integrative measure of smooth muscle cell relaxation and endothelial-dependent nitric oxide (NO) release (8–11), both of which can become disturbed in the presence of risk factors.

Nitric oxide is released by vascular endothelial cells and maintains low arterial tone at rest (12). Diminished bioavailability of NO impairs endothelium-dependent vasodilation and activates other mechanisms that may play an important role in the pathogenesis of atherosclerosis (13). In the L-arginine-NO pathway, NO synthase converts L-arginine to NO and L-citrulline (14). Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthase that can modulate NO production (15). A number of cells elaborate ADMA, and it is thought to be derived from proteins that have been post-translationally methylated and subsequently hydrolyzed (15). Plasma levels of ADMA and its biologically inactive structural isomer symmetrical dimethylarginine (SDMA) have been shown to be elevated in hypercholesterolemic animals (16,17) and humans (18). Clinical studies have demonstrated an association between increased plasma ADMA concentrations and hypertension (19,20). Miyazaki et al. (21) reported a direct relationship between plasma ADMA levels and carotid IMT in healthy humans. Böger et al. (18) showed that elevated plasma ADMA levels were associated with impaired endothelium-dependent brachial artery vasodilation in young hypercholesterolemic individuals. Together, these findings indicate an association of an elevated plasma ADMA level with hypertension and hyper-

From the *Department of Medicine, University of Tampere, Tampere, Finland; †Department of Clinical Pharmacology, University of Helsinki, Helsinki, Finland; ‡Department of Medicine, University of Turku, Turku, Finland; §Turku PET Centre, Turku University Central Hospital, Turku, Finland; and ‖Department of Clinical Physiology, University of Turku, Turku, Finland. Supported by the grants of the Academy of Finland, the Novo Nordisk Foundation, the Turku University Foundation, the Finnish Foundation for Cardiovascular Research, the Emil Aaltonen Foundation, the Sigrid Juselius Foundation, the Yrjö Jahnsson Foundation, the Leiras Scientific Foundation, the Ida Montin Foundation, and the Maud Kuistila Foundation.

Manuscript received June 20, 2000; revised manuscript received May 20, 2002, accepted June 27, 2002.

JACC Vol. 40, No. 7, 2002
October 2, 2002:1241–7

Abbreviations and Acronyms

ADMA = asymmetric dimethylarginine
BHT = borderline hypertension
BP = blood pressure
FH = familial hypercholesterolemia
HDL = high-density lipoprotein
HPLC = high-performance liquid chromatography
IMT = intima-media thickness
LDL = low-density lipoprotein
LV = left ventricle/ventricular
MBF = myocardial blood flow
NO = nitric oxide
OPA = o-pthalaldehyde
PET = positron emission tomography
ROI = region of interest
SDMA = symmetrical dimethylarginine

cholesterolemia, and suggest that ADMA represents a circulating marker of subclinical atherosclerosis.

To further examine the role of ADMA in early atherosclerosis, we measured plasma ADMA levels in young subjects with borderline hypertension (BHT) and familial hypercholesterolemia who participated in a noninvasive positron emission tomography (PET) study for the assessment resting and dipyridamole-induced MBF. We tested the hypothesis that these conditions are associated with elevated plasma ADMA levels compared with healthy control subjects, and examined whether high ADMA level is associated with hyperemic MBF responses.

METHODS

Subjects. The study population included 47 healthy controls (control group), 16 men with BHT (BHT group), and 14 men with familial hypercholesterolemia (FH group). The control group and BHT group were recruited from an ongoing intervention study for children, the Special Turku Coronary Risk Factor Intervention Project (STRIP)-baby study (22). Families in the STRIP-baby intervention study were recruited in the Turku area (a city of approximately 160,000 inhabitants in Finland). Those families with newborn babies born between March 1991 and May 1992 were contacted and recruited for the study. A total of 1,054 families with 1,062 infants agreed to participate (56.5% of the 1,880 eligible infants in the cohort). The subjects in the present study were fathers of these children. The inclusion criteria for healthy controls were: age <45 years, body mass index <27 kg/m^2, BP <150/90 mm Hg, nonsmoking, no history of diabetes, and no history of atherosclerotic disease. The men were recruited by a letter of invitation. Those fulfilling the criteria and living in the Turku area were recruited by a letter of invitation; on average 65% of those invited participated in PET studies.

The BHT group included healthy fathers with three to four BP measurement values during the previous two to three years (average follow-up 2.7 years) defined according to the Sixth Joint National Committee classification as high-normal (systolic BP 130 to 140 mm Hg or diastolic BP 85 to 89 mm Hg in all previous measurements). Otherwise, the inclusion criteria for the BHT group were the same as for controls. None of the subjects in the BHT group was receiving antihypertensive medication.

All eligible FH patients meeting the inclusion criteria were selected from the patient registry of Turku University Central Hospital and asked to participate by a letter of invitation. Subjects with a clinical history or evidence of coronary artery disease or other cardiac diseases, diabetes, systemic hypertension, or current smoking were excluded. Fourteen men (of 19 eligible men) with heterozygous FH volunteered for the study. All FH patients had at least one first-degree relative with hypercholesterolemia. The diagnosis of familial hypercholesterolemia was confirmed by deoxyribonucleic acid test in five men by identifying a known Finnish mutation using enzymatic amplification of genomic deoxyribonucleic acid by the polymerase chain reaction. Seven men had either clinical signs of tendon xanthomas or a positive finding in ultrasound scanning of the Achilles tendon (23) (distinctly thickened tendons, more than 10 mm). Three men had at least one first-degree relative with hypercholesterolemia and tendon xanthomata. Twelve patients had been receiving cholesterol-lowering medication for several years. Eight patients were receiving lovastatin, one was receiving lovastatin in combination with colestipol, one was receiving lovastatin in combination with acipimox, one was receiving bezafibrate, and one patient was receiving simvastatin in combination with cholestyramine. Two men were only undergoing diet therapy.

To rule out significant coronary heart disease, stress echocardiographic examinations were performed on all subjects with relatively low myocardial perfusion reserve values (the ratio of dipyridamole flow to basal flow <3). All these men had normal exercise capacity, were asymptomatic during the test, had no diagnostic ST-segment changes in electrocardiogram, and no wall motion disturbances either at rest or after the exercise test.

The study protocol was approved by the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital. Each subject gave a written informed consent.

PET study protocol. The study subjects were instructed to fast (including coffee, tea, and caffeine-containing soft-drinks) 12 h before the PET scan. Heart rate and electrocardiogram were monitored continuously during the studies. Blood pressure was monitored with an automatic oscillometric BP monitor (OMRON HEM-705C, Omron Corporation, Kyoto, Japan) during the PET study. Myocardial perfusion was measured twice, once at rest and once after administration of dipyridamole using PET and [^{15}O]-labelled tracers. To calculate myocardial perfusion noninvasively without arterial blood sampling, a mathematical model is used that corrects for the spillover of radioactivity both from the myocardium into the left ventricular (LV)

region of interest (ROI) and the blood into the myocardial ROI. The method requires the measurement of a time-activity curve in the LV chamber during the dynamic $[^{15}O]H_2O$ study and the measurement of the recovery coefficient of the LV ROI using a $[^{15}O]$-carbon monoxide scan and venous blood sampling (24).

In brief, the subjects were positioned supine in a 15-slice ECAT 931/08-12 tomograph (Siemens/CTI, Knoxville, Tennessee) with a measured axial resolution of 6.7 mm and in-plane resolution of 6.5 mm. The subjects inhaled $[^{15}O]$-carbon monoxide for 2 min (mean dose 3,400 ± 410 Mbq). After the inhalation, 2 min were allowed for carbon monoxide to combine with hemoglobin in red blood cells before a static scan for 4 min was started. During the scan period, three blood samples were drawn at 2-min intervals for blood radioactivity. A 10-min period was allowed for the radioactive decay of $[^{15}O]$-carbon monoxide before the flow measurements. Flow was measured at the baseline and 2 min after the end of intravenous administration of dipyridamole (0.56 mg per kg of body weight over a period of 4 min). A total of 1,650 ± 110 MBq of $[^{15}O]H_2O$ was injected intravenously during 2 min, and a dynamic scan was started for 6 min. Previous data have shown that resting and hyperemic MBF can be measured reproducibly with PET (25).

Calculation of regional blood flow. Regions of interest were placed on representative transaxial ventricular slices in each study covering anterior, septal, and lateral free wall of the LV. The ROIs were drawn on the images obtained at rest and copied to the images obtained after dipyridamole administration. Values of regional MBF (expressed in milliliters per gram of tissue per minute) were calculated according to the previously published method employing the single compartment model (26,27).

Qualitative analysis of the PET data did not reveal any regional differences in the distribution of blood flow. Therefore, in order to enhance accuracy and statistics of flow measurements, the average flow of the global left ventricular myocardium was calculated, and no detailed regional analysis was carried out.

Ultrasound imaging of carotid artery IMT. All measurements were performed by Acuson 128XP/10 (Acuson Inc., Mountain View, California) ultrasonography, using 7 MHz scanning frequency linear array transducer.

The common carotid artery far-wall IMT were measured from both sides in three different cardiac cycles approximately 10 mm distally from carotid bulb, and the values of 12 measurements were averaged, as described previously (28). In our laboratory the interobserver and intraobserver coefficients of variation of carotid IMT measurements were 5.2 ± 4.1% and 4.0 ± 3.2%, respectively (28). Intima-media thickness measurements were available from the majority of the study subjects: control subjects (43/47), BHT group (16/16), and FH group (7/14).

Serum lipoproteins. Venous blood samples were taken after a 12-h overnight fast during the same week as the PET study was performed. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were measured using standard enzymatic methods with a fully automated analyzer (Hitachi 704, Hitachi Ltd., Tokyo, Japan). High-density lipoprotein cholesterol was measured after polyethyleneglycol precipitation. The low-density lipoprotein (LDL) cholesterol concentration was calculated using the Friedewald formula (29). Apolipoprotein-B concentrations were measured by immunonephelometric method Behringen BNA (Behringswerke AG, Marburg, Germany).

Plasma ADMA, SDMA, and arginine. Plasma arginine and its dimethylated endogenous derivatives ADMA and SDMA were determined with a novel high-performance liquid chromatography (HPLC) method. Briefly, 200 μl serum was diluted with 160 μl of distilled water, and solution was mixed with 40 μl of internal standard (homoarginine, 400 μmol/l). Arginine, ADMA, and SDMA were then absorbed on a 100-mg Bond Elut silica solid phase extraction column (Varian, Harbor City, California), pretreated with 800 μl of methanol and with 800 μl of distilled water. After washing, arginine, ADMA, and SDMA were eluted from the SPE column with o-pthalaldehyde (OPA) derivatizing reagent. The OPA reagent was modified by adding acetonitrile and tetrahydrofuran (2:1:1). The stable OPA derivatives of arginine, ADMA, and SDMA were then separated with HPLC on an Waters Bondapak phenyl column (5 μm, 150 × 4.6 mm) with 10 mmol/l phosphate buffer containing 870 mg/l of hexanesulphonic acid as a mobile phase. Fluorescence was monitored at 328 nm (exitation) and 445 nm (emission). The HPLC equipment consisted of an LKB Pharmacia 2248 pump, a Hewlett-Packard 1050 autosampler, and a Shimadzu RF-551 fluorescence detector. Total imprecision (coefficient of variation %) for ADMA, SDMA, and arginine were not more than 12% as studied by repeated analyses of a pooled sample during the analyses of the actual samples.

Statistical methods. The results are expressed as mean ± SD. The distribution for triglycerides was skewed; therefore, the values were $\log_{10}$-transformed before analysis. Data comparison among three groups was based on analysis of variance with respective all pairwise multiple comparison post-hoc analysis using the Bonferroni method. Student *t* tests were used for data comparison between two groups. The univariate association between dipyridamole flow and plasma ADMA concentration was assessed by correlation analysis. Multivariate determinants of flow variables (baseline and dipyridamole flow) were assessed by stepwise linear regression technique including ADMA, age, study group, serum lipids, and BP as covariates, with $p < 0.15$ as model entry criteria. All statistical tests were performed using SAS software (SAS Institute, Cary, North Carolina).

RESULTS

The characteristics of study subjects are shown in Table 1. The FH group had higher serum LDL, apolipoprotein B,

JACC Vol. 40, No. 7, 2002
October 2, 2002:1241–7

Table 1. Characteristics of Study Subjects

	Controls	BHT Group	FH Group
No.	47	16	14
Age (yrs)	35 ± 4	37 ± 4†	31 ± 8*
BMI (kg/m^2)	24.6 ± 2.2	26.1 ± 2.5	26.2 ± 2.9
LDL-c (mmol/l)	3.18 ± 1.10	3.55 ± 0.89†	6.00 ± 1.80*
HDL-c (mmol/l)	1.24 ± 0.28	1.11 ± 0.17	0.96 ± 0.17*
Triglycerides (mmol/l)	1.06 ± 0.53	1.95 ± 0.98*	1.32 ± 0.39
Apolipoprotein B (g/l)	0.87 ± 0.28	1.13 ± 0.28*†	1.49 ± 0.35*
Apolipoprotein A-I (g/l)	1.54 ± 0.21	1.48 ± 0.18	1.35 ± 0.16*
ADMA (μmol/l)	0.43 ± 0.12	0.59 ± 0.13*†	0.44 ± 0.19
SDMA (μmol/l)	0.33 ± 0.08	0.33 ± 0.06	0.34 ± 0.12
Arginine (μmol/l)	118 ± 31	107 ± 31	135 ± 25
Systolic BP (mm Hg)	114 ± 12	137 ± 22*†	122 ± 8
Diastolic BP (mm Hg)	63 ± 8	82 ± 10*†	63 ± 10
Carotid IMT‡ (mm)	0.56 ± 0.07	0.75 ± 0.07*†	0.65 ± 0.11*

*$p < 0.05$ vs. controls; †$p < 0.05$ vs. FH group; ‡Controls (n = 43), BHT group (n = 16), FH group (n = 7).

ADMA = asymmetric dimethyl arginine; BHT = borderline hypertension; BMI = body mass index; BP = blood pressure; FH = familial hypercholesterolemia; HDL-c = high-density lipoprotein-cholesterol; IMT = intima media thickness; LDL-c = low-density lipoprotein-cholesterol; SDMA = symmetrical dimethyl arginine.

triglycerides, and carotid IMT compared with healthy controls but comparable ADMA, SDMA, and arginine levels. The BHT group had higher BP values, apolipoprotein B, triglycerides, and carotid IMT compared with control subjects and significantly higher ADMA levels. The levels of SDMA and arginine were similar between the three groups.

Basal flow was similar in the BHT group and controls (0.83 ± 0.21 ml/min/g vs. 0.80 ± 0.16 ml/min/g, p = NS), but the BHT group had significantly lower dipyridamole flow (2.85 ± 1.20 ml/min/g vs. 3.69 ± 1.68 ml/min/g, $p < 0.05$) and, therefore, lower flow reserve (3.47 ± 1.23 vs. 4.66 ± 2.30, $p < 0.05$). Compared with controls, the FH group had non-significantly higher basal flow (0.93 ± 0.25 ml/min/g vs. 0.80 ± 0.16 ml/min/g, p = NS) and lower dipyridamole flow (3.21 ± 1.65 vs. 3.69 ± 1.68, p = NS), which resulted in significantly lower flow reserve in the FH group (3.48 ± 1.61 vs. 4.66 ± 2.30, $p < 0.05$).

There was a weak inverse correlation between ADMA and dipyridamole flow ($r = -0.22$, $p < 0.05$) (Fig. 1). To study the association of very high ADMA levels on flow variables, we compared those subjects in the highest quintale of ADMA distribution (n = 15, ADMA >0.58 μmol/l) to others (n = 62). Subjects with high ADMA levels had significantly higher carotid IMT (0.60 ± 0.10 mm vs. 0.67 ± 0.14 mm, p = 0.03), serum triglycerides (1.79 ± 1.02 mmol/l vs. 1.17 ± 0.56 mmol/l, p = 0.04), and lower dipyridamole flow (2.60 ± 1.12 ml/min/g vs. 3.63 ± 1.65 ml/min/g, p = 0.03). The association between high ADMA levels and low dipyridamole flow was also observed when the analysis was restricted to healthy control subjects (high ADMA: n = 5 vs. others, n = 42; dipyridamole flow: 2.35 ± 1.24 vs. 3.85 ± 1.66, p = 0.05). A nonsignificant trend toward lower dipyridamole flow with high ADMA levels was also seen in the FH group (high ADMA, n = 4 vs. others, n = 10; dipyridamole flow 2.51 ± 2.37 vs. 3.50 ± 1.3, p = 0.33).

Multivariate determinants of flow variables. Determinants of flow variables were studied with multivariate stepwise regression models including age, study group,

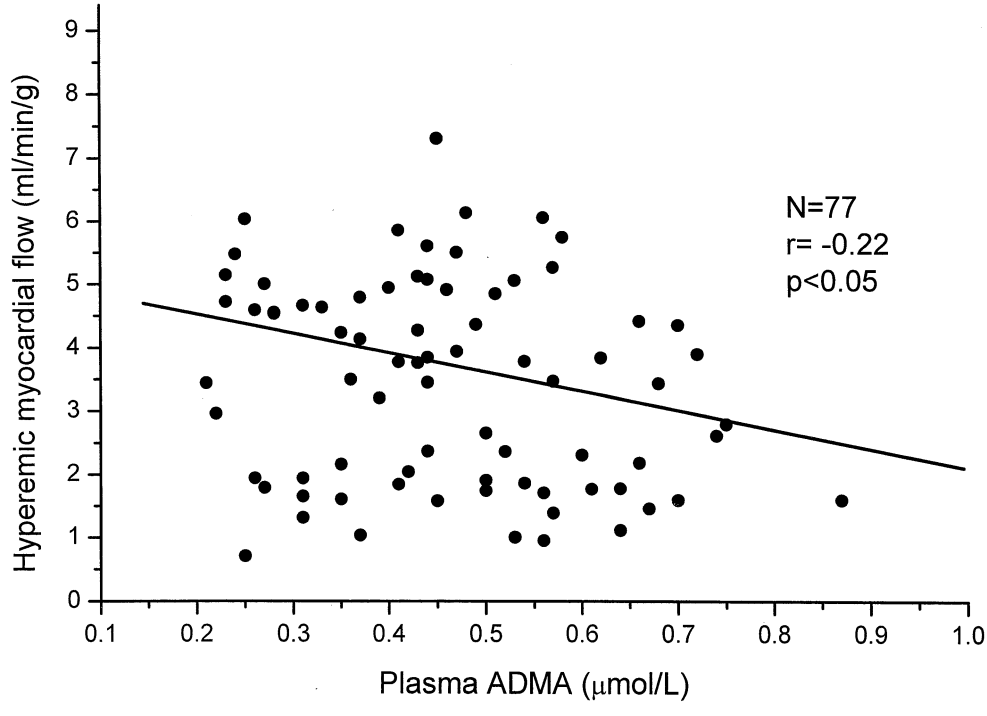

Figure 1. Scatterplot showing the correlation between plasma asymmetric dimethylarginine (ADMA) and hyperemic myocardial blood flow in pooled data.

Table 2. Multivariate Determinants of Myocardial Flow Variables

	Basal Flow		Dipyridamole Flow	
	F	p Value	F	p Value
	$R^2 = 0.10$		$R^2 = 0.15$	
Age	4.3	0.04	4.2	0.04
Study group	·	·	·	·
ADMA (high-low)	2.7	0.1	5.2	0.03
LDL-c	·	·	·	·
HDL-c	·	·	·	·
Triglycerides	·	·	·	·
Apolipoprotein B	·	·	2.5	0.12
Diastolic BP	3.3	0.07	·	·
Systolic BP	·	·	·	·

· = not significant ($p > 0.15$); ADMA = asymmetric dimethylarginine; BP = blood pressure; HDL-c = high-density lipoprotein cholesterol; LDL-c = low-density lipoprotein cholesterol.

ADMA, apolipoprotein B, LDL cholesterol, HDL cholesterol, triglycerides, and BP as covariates (Table 2). Significant correlates of basal flow included age and diastolic BP. Determinants of dipyridamole flow included age, ADMA, and apolipoprotein B.

DISCUSSION

The present study shows that young adults with BHT have significantly higher plasma ADMA levels and reduced hyperemic MBF compared with normotensive control subjects. Multivariate analysis adjusted for the effects of study group and other risk variables indicated that high plasma ADMA level was related to reduced dipyridamole flow. Together, these findings suggest that high ADMA levels are associated with BP elevation, and may partly explain reduced hyperemic MBF responses in healthy subjects with BHT. Interestingly, the association between high ADMA levels and low dipyridamole flow was also observed when the analysis was restricted to healthy control subjects, suggesting that the ADMA level may modulate myocardial hyperemic responses also under normal physiologic conditions.

ADMA and hypertension. Because methylarginines are, in part, eliminated by renal excretion, they accumulate in the plasma of patients with end-stage renal failure (30–32), and it has been suggested that the accumulation of ADMA contributes to the development of hypertension in these patients (33). Elevated ADMA levels may also play a role in the development of essential hypertension. Surdacki et al. (19) observed that men with newly diagnosed and untreated hypertension had increased plasma ADMA levels and depressed systemic NO formation. Observations showing increased plasma levels of ADMA in young hypertensive children (20) and in women with pregnancy-induced hypertension (34,35) also indicate that elevations in ADMA levels may play a role in the early stages of hypertension. Borderline hypertension is a strong risk factor for established hypertension, and this condition offers a useful model for "prehypertension" to examine the early metabolic manifestations related to elevated BP levels without the confounding effects mediated by other risk factors commonly clustering with advanced stages of hypertension (28). The finding of the present study showing high ADMA levels in subjects with BHT is in line with previous observations and supports the idea that high ADMA level may be a risk factor for BP elevation.

ADMA and hypercholesterolemia. Contrary to the study by Böger et al. (18), we could not demonstrate an association between hypercholesterolemia and ADMA. Similarly, Miyazaki et al. (21) were unable to show a significant relationship between serum cholesterol concentration and ADMA in middle-aged subjects. Also, a recent study by Cardinale et al. (36) in subjects with established atherosclerosis did not observe any association between cholesterol concentration and ADMA. Differences in study populations may explain the discrepant results. For example, the majority of our FH patients in the current study were receiving lipid-lowering therapy. Furthermore, FH patients have a single-gene defect and may differ from subjects with polygenic hypercholesterolemia. These observations, however, do not necessarily imply that ADMA levels are irrelevant to vascular reactivity or atherosclerotic risk. In the study by Miyazaki et al. (21), plasma ADMA level was significantly associated with elevated BP and increased carotid artery IMT, a marker of subclinical atherosclerosis. Furthermore, in the study by Cardinale et al. (36), elevated plasma ADMA level was associated with increased atherosclerosis in the thoracic aorta. In the present study high ADMA level was associated with dipyridamole flow independently of the study group, and a trend towards lower dipyridamole flow with high ADMA level was also seen within the FH group.

Effect of dipyridamole. Dipyridamole causes accumulation of intracellular adenosine that has a direct vasodilatory effect on smooth muscle cells via purinergic vascular receptors (37). Part of the effects of dipyridamole may be mediated by vascular endothelium because its effects can be reduced by inhibiting NO synthesis (10). Furthermore, it has been suggested that an increase in shear stress due to increased flow after dipyridamole administration will induce the release of vasodilating substances from endothelial cells and elicit more prominent vasodilation in the vessels with preserved endothelial function (38). Recent study showed that approximately one-third of the adenosine-induced myocardial hyperemic blood flow response is blocked by simultaneous administration of NO synthase inhibitor N^G-nitro-L-arginine (39). Therefore, the myocardial flow response to dipyridamole may be regarded as an integrated measure of vascular smooth muscle relaxation and endothelial function.

Measurement of ADMA. We used HPLC and OPA derivatization to measure plasma ADMA. The method produced sharp HPLC peaks, and the variability of repeated measurements was low. The plasma levels in control subjects averaged 0.43 ± 0.12 μmmol/l (range 0.21 to 0.66 μmmol/l). These values are similar to those reported in

healthy controls in several previous studies (21,30,31,40) but slightly higher than in some reports (41,42) and lower than in others (18,19) that have reported ADMA levels approximately 1.0 μmmol/l in healthy controls. The levels of plasma ADMA in subjects with BHT in the current study were similar than previously reported in dialysis patients (30) or patients with preeclampsia (35). The variable results regarding plasma ADMA levels are likely explained by differences in methodology. Serum ADMA levels may be determined employing a variety of methods including HPLC-ultraviolet or HPLC with fluorescence detection after a derivatization step, capillary electrophoresis with laser-induced fluorescence detection, and HPLC tandem mass spectrometry. At present no interlaboratory comparisons have been reported. Consequently, the reasons for discrepancy in ADMA levels obtained with different methods have not been clarified.

Study limitations. The present study examined the relationships between ADMA and myocardial reactivity in a cross-sectional setting. A more ideal approach would be prospective study of subjects before and after therapeutic interventions aimed at altering plasma ADMA levels. As there is currently no effective means to reduce plasma ADMA levels, such intervention study would not be possible to perform. Therefore, these data must be interpreted carefully, as they do not necessarily indicate a causal relationship between elevated ADMA levels and reduced hyperemic MBF in hypertension; ADMA may play a causal role or be simply a marker of the disorder. We have studied only those volunteers approached and willing to consent to studies on the effects of risk factors on arterial physiology, and, therefore, some selection bias may be present. Only men were investigated; therefore, it is unknown whether the same results can be extrapolated to female subjects. In the FH group, 12 patients had been receiving cholesterol-lowering medication for several years. Despite this, their cholesterol levels were elevated. It is possible, however, that the results obtained in these medically treated patients may underestimate the impact of FH on myocardial reactivity. It is also unknown whether long-term lipid-lowering therapy influences ADMA concentrations.

Conclusions. We conclude that plasma ADMA concentration correlates with dipyridamole-induced vasodilatory function in young males, independently of BP elevation and hypercholesterolemia. Subjects with BHT have significantly increased plasma ADMA levels, which may partly explain impaired myocardial reactivity in this condition.

Acknowledgments

The authors thank the personnel of the Turku PET Centre for their technical assistance.

Reprint requests and correspondence: Dr. Olli T. Raitakari, Turku PET Centre, Turku University Central Hospital, FIN-20520 Turku, Finland. E-mail: olli.raitakari@utu.fi.

REFERENCES

1. Dayanikli F, Grambow D, Muzik O, Mosca L, Rubenfire M, Schwaiger M. Early detection of abnormal coronary flow reserve in asymptomatic men at high risk for coronary artery disease using positron emission tomography. Circulation 1994;90:808–17.
2. Yokoyama I, Ohtake T, Momomura S, Nishikawa J, Sasaki Y, Omata M. Reduced coronary flow reserve in hypercholesterolemic patients without overt coronary stenosis. Circulation 1996;94:3232–8.
3. Pitkänen O-P, Nuutila P, Raitakari OT, et al. Coronary flow reserve in young men with familial combined hypercholesterolemia. Circulation 1999;99:1678–84.
4. Pitkänen O-P, Nuutila P, Raitakari OT, et al. Coronary flow reserve is reduced in young men with insulin-dependent diabetes mellitus. Diabetes 1998;47:248–54.
5. Laine H, Raitakari OT, Niinikoski H, et al. Early impairment of coronary flow reserve in young men with borderline hypertension. J Am Coll Cardiol 1998;32:147–53.
6. Raitakari OT, Pitkänen O-P, Lehtimäki T, et al. In vivo LDL oxidation relates to coronary reactivity in young men. J Am Coll Cardiol 1997;30:97–102.
7. Raitakari OT, Toikka JO, Ahotupa M, et al. Reduced myocardial flow reserve relates to increased carotid intima-media thickness in healthy young men. Atherosclerosis 2001;156:469–75.
8. Lupi A, Buffon A, Finocchiaro ML, Conti E, Masera A, Crea F. Mechanisms of adenosine-induced epicardial coronary artery dilatation. Eur Heart J 1997;18:614–7.
9. Rubanyi G, Vanhoutte PM. Endothelial-removal decreases relaxations of canine coronary arteries caused by adrenergic agonists and adenosine. J Cardiovasc Pharmacol 1985;7:139–44.
10. Smits P, Williams SB, Lipson DE, Banitt P, Rongen GA, Creager MA. Endothelial release of nitric oxide contributes to the vasodilator effect of adenosine in humans. Circulation 1995;92:2135–41.
11. Davis CA, Sherman AJ, Yaroshenko Y, et al. Coronary vascular responsiveness to adenosine is impaired additively by blockade of nitric oxide synthesis and a sulfonylurea. J Am Coll Cardiol 1998;31:816–22.
12. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524–6.
13. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801–9.
14. Hecker M, Sessa WC, Harris HJ, Anggard EE, Vane JR. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: cultured endothelial cells recycle L-citrulline to L-arginine. Proc Natl Acad Sci USA 1990;87:8612–6.
15. Leiper J, Vallance P. Biological significance of endogenous methylargines that inhibit nitric oxide synthase. Cardiovasc Res 1999;43:542–8.
16. Bode-Böger SM, Böger RH, Kienke S, Junker W, Frölich JC. Elevated L-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary L-arginine in hypercholesterolemic rabbits. Biochem Biophys Res Commun 1996;219:598–603.
17. Böger RH, Bode-Böger SM, Sydow K, Heistad DD, Lentz SR. Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in monkeys with hyperhomocysteinemia or hypercholesterolemia. Arterioscler Thromb Vasc Biol 2000;20:1557–64.
18. Böger RH, Bode-Böger SM, Szuba A, et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998;98:1842–7.
19. Surdacki A, Nowicki M, Sandmann J, et al. Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. J Cardiovasc Pharmacol 1999;33:652–8.
20. Goonasekera CD, Rees DD, Woolard P, Frend A, Shah V, Dillon MJ. Nitric oxide synthase inhibitors and hypertension in children and adolescents. J Hypertens 1997;15:901–9.
21. Miyazaki H, Matsuoka H, Cooke JP, et al. Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. Circulation 1999;99:1141–6.
22. Lapinleimu H, Viikari J, Jokinen E, et al. A prospective randomized trial in 1062 infants of a reduced saturated fat and cholesterol diet. Lancet 1995;345:471–6.

23. Koivunen-Niemelä T, Alanen A, Viikari J. Sonography of the Achilles tendon in hypercholesterolemia. J Intern Med 1993;234:401–5.
24. Iida H, Rhodes C, de Silva R, et al. Use of the left ventricular time-activity curve as a noninvasive input function in dynamic oxygen-15-water positron emission tomography. J Nucl Med 1992;33:1669–77.
25. Nagamachi S, Czernin J, Kim AS, et al. Reproducibility of measurements of regional and hyperemic myocardial blood flow assessed with PET. J Nucl Med 1996;37:1626–31.
26. Iida H, Kanno I, Takahashi A, et al. Measurement of absolute myocardial blood flow with H215O and dynamic positron-emission tomography: strategy for quantification in relation to the partial-volume effect. Circulation 1988;78:104–15.
27. Iida H, Takahashi A, Tamura Y, Ono Y, Lammertsma A. Myocardial blood flow: comparison of oxygen-15-water bolus injection, slow infusion and oxygen-15-carbon dioxide slow inhalation. J Nucl Med 1995;36:78–85.
28. Toikka JO, Laine H, Ahotupa M, et al. Increased arterial intima-media thickness and in vivo LDL oxidation in young men with borderline hypertension. Hypertension 2000;36:929–33.
29. Friedewald WT, Levy R, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
30. Anderstam B, Katzarski K, Bergström J. Serum levels of NG, NG-dimethyl-L-arginine, a potential endogenous nitric oxide inhibitor in dialysis patients. J Am Soc Nephrol 1997;8:1437–42.
31. MacAllister RJ, Rambausek MH, Vallance P, Williams D, Hoffmann KH, Ritz E. Concentration of dimethyl-L-arginine in the plasma of patients with end-stage renal failure. Nephrol Dial Transplant 1996; 11:2449–52.
32. Schmidt RJ, Domico J, Samsell LS, et al. Indices of activity of the nitric oxide system in hemodialysis patients. Am J Kidney Dis 1999;34:228–34.
33. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992;339:572–5.
34. Holden DP, Fickling SA, Whitley GS, Nussey SS. Plasma concentrations of asymmetric dimethylarginine, a nature inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. Am J Obstet Gynecol 1998;178:551–6.
35. Pettersson A, Hedner T, Milsom I. Increased circulating concentrations of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. Acta Obstet Gynaecol Scand 1998;77:808–13.
36. Cardinale CP, Fard A, Eisenberg MS, Kazanowski MA, DiTullio MR, Homma S. Elevated plasma level of asymmetric dimethylarginine (ADMA) is associated with atherosclerotic disease of the thoracic aorta (abstr). J Am Coll Cardiol 2001;37 Suppl A:648A.
37. Opie LH. Oxygen supply, coronary flow. In: Opie LH, editor. The Heart. 2nd ed. New York, NY: Raven Press, 1991:277–300.
38. Rubanyi G, Romero J, Vanhoutte P. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986;240: H1145–9.
39. Buus NH, Bottcher M, Hermansen F, Sander M, Nielsen TT, Mulnavy MJ. Influence of nitric oxide synthase and adrenergic inhibition on adenosine-induced myocardial hyperemia. Circulation 2001;104:2305–10.
40. Pi J, Kumagai Y, Sun G, Shimojo N. Improved method for simultaneous determination of L-arginine and its mono- and dimethylated metabolites in biological samples by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 2000;742:199–203.
41. Causse E, Siri N, Arnal JF, et al. Determinations of asymmetrical dimethylarginine by capillary electrophoresis-laser-induced fluorescence. J Chromatogr B Biomed Sci Appl 2000;741:77–83.
42. Meyer J, Richter N, Hecker M. High-performance liquid chromatographic determination of nitric oxide synthase-related arginine derivatives in vitro and in vivo. Anal Biochem 1997;247:11–6.